

CONJUGATES AND USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of United States provisional
5 application serial no. 60/468,330 filed May 6, 2003.

FIELD OF THE INVENTION

This invention relates to a method for treating lupus erythematosus.
More particularly, ligands that bind to activated macrophages or other stimulated
10 immune cells are conjugated with an immunogen, a cytotoxin, or another agent for
altering macrophage function, or the function of other stimulated immune cells, for
administration to a patient for treatment of lupus erythematosus.

BACKGROUND AND SUMMARY OF THE INVENTION

15 The mammalian immune system provides a means for the recognition
and elimination of foreign pathogens. While the immune system normally provides a
line of defense against foreign pathogens, there are many instances where the immune
response itself is involved in the progression of disease. Exemplary of diseases
caused or worsened by the host's own immune response are autoimmune diseases
20 such as multiple sclerosis, lupus erythematosus, psoriasis, pulmonary fibrosis, and
rheumatoid arthritis and diseases in which the immune response contributes to
pathogenesis such as atherosclerosis, inflammatory diseases, osteomyelitis, ulcerative
colitis, Crohn's disease, and graft versus host disease often resulting in organ
transplant rejection.

25 Macrophages are generally the first cells to encounter foreign
pathogens, and accordingly, they play an important role in the immune response.
Activated macrophages nonspecifically engulf and kill foreign pathogens within the
macrophage by hydrolytic and oxidative attack resulting in degradation of the
pathogen. Peptides from degraded proteins are displayed on the macrophage cell
30 surface where they can be recognized by T cells, and they can directly interact with
antibodies on the B cell surface, resulting in T and B cell activation and further
stimulation of the immune response.

Lupus erythematosus, including both systemic and cutaneous forms of the disease, is an autoimmune disease of unknown etiology in which tissues are damaged by autoantibodies and immune complexes. There is evidence that there is a genetic predisposition to lupus erythematosus, and the prevalence of this disease is about eight times higher in women of child-bearing age than in men. There are also environmental factors that may trigger episodes of lupus erythematosus including ingestion of some foods and exposure to certain chemicals and ultraviolet light. The prevalence of systemic lupus erythematosus in urban areas is about 15-50 for a population of 100,000.

Abnormal immune responses that are known to be involved in systemic lupus erythematosus include aberrant regulation of self-reactive T and B lymphocytes resulting in increased activity of these cells. This T and B cell hyperactivity permits sustained production of autoantibodies and immune complexes. Some of the autoantibodies produced induce the symptoms of lupus erythematosus by binding directly to self-antigens and others attach to cell membranes via charge interactions or via cross-reactivity with cell or tissue components. Accordingly, not only is the etiology of lupus erythematosus unknown, but, except for the involvement of T and B cells in lupus erythematosus, the involvement of other immune cells in lupus erythematosus is not well-understood.

There are multiple clinical manifestations of lupus erythematosus including musculoskeletal manifestations, such as myalgias and intermittent arthritis, cutaneous manifestations, including erythematosus rashes, renal manifestations such as deposition of immunoglobulins in the glomeruli and clinical nephritis, nervous system manifestations such as mild cognitive dysfunction, headaches, and seizures, vascular and hematologic symptoms including thrombosis, anemia, hemolysis, leukopenia, and thrombocytopenia, cardiopulmonary manifestations such as pericarditis, arrhythmias, pleurisy, and pleural effusions, gastrointestinal manifestations such as nausea and diarrhea, and ocular manifestations such as retinal vasculitis. Treatments for lupus erythematosus include the use of glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs) and daily treatment with antimalarials, such as hydroxychloroquine, is used to improve skin lesions. However, glucocorticoids and NSAIDs, in particular, have undesirable side effects and attempts are made to limit their use to minimize undesirable side effects. Therefore, the

available therapies for the treatment of the multiple clinical manifestations of lupus erythematosus have undesirable side effects highlighting the need for new therapies to treat this disease.

The present invention is directed to a method for treating lupus erythematosus by killing or inhibiting the function of activated macrophages or other stimulated immune cells. In accordance with one embodiment, the host immune response is redirected to activated macrophages by "labeling" activated macrophages with an immunogen. In another embodiment, to promote killing or to inhibit the function of activated macrophages, ligands that bind preferentially to activated macrophages compared to resting macrophages are conjugated to a cytotoxin for direct killing or inhibition of the function of activated macrophages. In yet another embodiment, ligands that bind preferentially to activated macrophages are conjugated to another compound capable of altering the function of activated macrophages to inhibit activity of the activated macrophage population. Ligands that can be used in the conjugates of the present invention include those that bind to receptors preferentially expressed or presented on activated macrophages compared to resting macrophages, such as the folate receptor, or ligands such as monoclonal antibodies directed to cell surface markers preferentially expressed on activated macrophages.

In one embodiment, a method of treating lupus erythematosus is provided. The method comprises the step of administering to a patient suffering from lupus erythematosus an effective amount of a composition comprising a conjugate of the general formula L-X where the group L comprises a ligand capable of binding to activated macrophages and the group X comprises an immunogen, a cytotoxin, or another compound capable of altering macrophage function.

In another embodiment, a method of treating lupus erythematosus is provided. The method comprises the step of administering to a patient suffering from lupus erythematosus an effective amount of a composition comprising a conjugate of the general formula L-X where the group L comprises a vitamin, or a vitamin-receptor binding analog or derivative thereof, and the group X comprises an immunogen, a cytotoxin, or another compound capable of altering macrophage function.

In another embodiment, a use of a conjugate of the general formula L-X where the group L comprises a ligand capable of binding to activated

macrophages and the group X comprises an immunogen, a cytotoxin, or another compound capable of altering macrophage function, in the manufacture of a medicament for treating lupus erythematosus. In another embodiment, L can comprise a vitamin, or a vitamin-receptor binding analog or derivative thereof.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the percentage uptake of EC20 in the organs of lupus prone MRL/lpr mice (bar on left for each organ) versus control mice (bar on right for each organ).

10

Fig. 2 shows a photomicrograph of lupus prone MRL/lpr mice that have either been injected with the ligand conjugates of the present invention (mouse on the left) or have been injected with PBS (control; mouse on the right).

Fig. 3 shows a survival curve for lupus prone MRL/lpr mice that have been injected with ligand conjugates used in the method of the present invention, folate-FITC (open diamonds), or have been injected with PBS (control; closed diamonds).

15

DETAILED DESCRIPTION OF THE INVENTION

A method is provided in accordance with the present invention for treating lupus erythematosus. Lupus erythematosus can be treated in accordance with this invention by administering an effective amount of a composition of the formula L-X, wherein L comprises a ligand capable of binding to an activated macrophage and wherein the group X comprises an immunogen, a cytotoxin, or another compound, such as a cytokine, capable of altering macrophage function. Such macrophage targeting conjugates, when administered to a patient suffering from lupus erythematosus, can work to concentrate and associate the conjugated cytotoxin, immunogen, or other compound capable of altering macrophage function with a population of activated macrophages to kill the activated macrophages or alter macrophage function. Elimination or deactivation of the activated macrophage population can work to eliminate or reduce the pathogenesis characteristic of lupus erythematosus. The conjugate is typically administered parenterally as a composition comprising the ligand conjugate and a pharmaceutically acceptable carrier therefor.

30

Conjugate administration is typically continued until symptoms of the disease state are reduced or eliminated.

The method of the present invention can be used for both human clinical medicine and veterinary applications. Thus, the patient afflicted with lupus erythematosus, or a similar disease state, can be a human, or in the case of veterinary applications, can be a laboratory, agricultural, domestic or a wild animal. The conjugates administered in accordance with the methods of this invention are preferably administered parenterally to the patient suffering from lupus erythematosus, for example, intradermally, subcutaneously, intramuscularly, intraperitoneally, or intravenously. Alternatively, the conjugates can be administered to the patient by other medically useful procedures and effective doses can be administered in standard or prolonged release dosage forms, such as a slow pump. The therapeutic method of the present invention may be used alone, or in combination with other therapeutic methods, including those recognized for the treatment of lupus erythematosus.

In accordance with the present invention, the ligand conjugates can be formed from a wide variety of ligands, including any ligand capable of binding to a receptor expressed or presented on the surface of activated macrophages that is not expressed/presented or is not present in significant amounts on the surface of resting macrophages. Such ligands include N-formyl peptides (e.g., f-Met-Leu-Phe), high mobility group factor 1 protein (HMGB1), hyaluronan fragments, HSP-70, toll-like receptor ligands, scavenger receptor ligands, co-receptors for antigen presentation, ligands that bind to the CD68, BER-MAC3, RFD7, CD4, CD14, and HLA-D markers on activated macrophages, antibodies, or fragments thereof, that bind preferentially to activated macrophages, and vitamins or receptor-binding vitamin analogs/derivatives. The ligand conjugates are capable of preferentially binding to activated macrophages compared to resting macrophages due to preferential expression of the receptor for the ligand on activated macrophages compared to resting macrophages.

Acceptable vitamin moieties that can be used as ligands in accordance with the invention include niacin, pantothenic acid, folic acid, riboflavin, thiamine, biotin, vitamin B₁₂, and the lipid soluble vitamins A, D, E and K. These vitamins, and their receptor-binding analogs and derivatives, constitute the targeting entity that can be coupled with an immunogen, a cytotoxin, or another compound capable of altering

macrophage function, to form the ligand conjugates for use in accordance with the invention. Preferred vitamin moieties include folic acid, biotin, riboflavin, thiamine, vitamin B₁₂, and receptor-binding analogs and derivatives of these vitamin molecules, and other related vitamin receptor-binding molecules (see U.S. Patent No. 5,688,488, incorporated herein by reference).

In the ligand conjugates of the general formula L-X in accordance with an embodiment of the present invention, the group L is a ligand capable of binding to activated macrophages as compared to resting macrophages as described above. In one embodiment the activated macrophage binding ligand is folic acid, a folic acid analog or other folate receptor-binding molecules. In another embodiment the activated macrophage binding ligand is a specific monoclonal or polyclonal antibody or Fab or scFv (i.e., a single chain variable region) fragments of an antibody capable of preferential binding to activated macrophages as compared to resting macrophages.

Activated macrophages express a 38 kD GPI-anchored folate receptor that binds folate and folate-derivatized compounds with subnanomolar affinity (i.e., < 1 nM). Importantly, covalent conjugation of small molecules, proteins, and even liposomes to folic acid does not alter the vitamin's ability to bind the folate receptor. Because most cells use an unrelated reduced folate carrier to acquire the necessary folic acid, expression of the folate receptor is restricted to a few cell types. With the exception of kidney and placenta, normal tissues express low or nondetectable levels of the folate receptor. However, many malignant tissues, including ovarian, breast, bronchial, and brain cancers express significantly elevated levels of the receptor. In fact, it is estimated that 95% of all ovarian carcinomas overexpress the folate receptor. Also, it has recently been reported that the folate receptor β , the nonepithelial isoform of the folate receptor, is expressed on activated, but not resting synovial macrophages. Thus, Applicants have found that folate-immunogen conjugates can be used in the method of the present invention to redirect the host immune response in animals with lupus erythematosus to activated macrophages to deplete macrophages and reduce the symptoms of disease.

Conjugates wherein the group L is folic acid, a folic acid analog, or another folic acid receptor-binding ligand are described in detail in U.S. Patent No. 5,688,488, incorporated herein by reference. That patent, as well as related U.S. Patents Nos. 5,416,016 and 5,108,921, each incorporated herein by reference,

describe methods and examples for preparing conjugates useful in accordance with the present invention. The present macrophage-targeted ligand conjugates can be prepared and used following general protocols described in those patents.

Folinic acid, pteronic acid, pteropolylglutamic acid, and folate receptor-binding pteridines such as tetrahydropterins, dihydrofolates, tetrahydrofolates, and their deaza and dideaza analogs can also be used in accordance with the invention. The terms "deaza" and "dideaza" analogs refers to the art-recognized folate analogs having a carbon atom substituted for one or two nitrogen atoms in the naturally occurring folic acid structure. For example, the deaza analogs include the 1-deaza, 3-deaza, 5-deaza, 8-deaza, and 10-deaza analogs. The dideaza analogs include, for example, 1,5 dideaza, 5,10-dideaza, 8,10-dideaza, and 5,8-dideaza analogs. The foregoing are folate analogs or derivatives and can bind to folate receptors. Other folate analogs or derivatives useful in accordance with the invention are the folate receptor-binding analogs aminopterin, amethopterin (methotrexate), N¹⁰-methylfolate, 2-deamino-hydroxyfolate, deaza analogs such as 1-deazamethopterin or 3-deazamethopterin, and 3'5'-dichloro-4-amino-4-deoxy-N¹⁰-methylpteroylglutamic acid (dichloromethotrexate). Another folate analog is folic acid containing a glutamic acid residue in the D configuration (folic acid normally contains one glutamic acid in the L configuration linked to pteronic acid).

The binding site for the ligand can include receptors for any ligand molecule, or a derivative or analog thereof, preferentially expressed/presented on the surface of activated macrophages other stimulated immune cells. A surface-presented protein preferentially expressed by activated macrophages is a receptor that is either not present or is present at insignificant concentrations on resting macrophages providing a means for preferential binding to activated macrophages. Accordingly, any receptor that is upregulated on activated macrophages compared to resting macrophages, or which is not expressed/presented on the surface of resting macrophages, or any receptor that is not expressed/presented on the surface of resting macrophages in significant amounts could be used for targeting. In one embodiment the site that binds the ligand conjugates used in accordance with the present invention is a vitamin receptor, for example, the folate receptor, which binds folate, or an analog or derivative thereof.

In accordance with the invention the ligand conjugates can bind with high affinity to receptors on activated macrophages. The high affinity binding can be inherent to the ligand or the binding affinity can be enhanced by the use of a chemically modified ligand (i.e., an analog or a derivative) or by the particular
5 chemical linkage, in the ligand conjugate, between the ligand and the immunogen, cytotoxin, or other compound capable of altering macrophage function.

The chemical linkage in the ligand conjugate between the ligand and the immunogen, cytotoxin, or other compound capable of altering macrophage function can be a direct linkage or can be through an intermediary linker. If present,
10 an intermediary linker can be any biocompatible linker known in the art. Typically, the linker comprises about 1 to about 30 carbon or other atoms, more typically about 2 to about 20 atoms. Lower molecular weight linkers (i.e., those having an approximate molecular weight of about 30 to about 300) are typically employed.

Generally, any manner of forming a conjugate between the ligand and
15 the immunogen, between the ligand and the cytotoxin, between the ligand and the compound capable of altering macrophage function, between a linker and the ligand, or between a linker and the immunogen, cytotoxin, or other compound capable of altering macrophage function can be utilized in accordance with the present invention. With or without a linker, the complex can be formed by conjugation of the
20 components of the conjugate, for example, through hydrogen, ionic, or covalent bonds. Covalent bonding of the components of the conjugate can occur, for example, through the formation of amide, ester, disulfide, or imino bonds between acid, aldehyde, hydroxy, amino, sulfhydryl, or hydrazo groups. Also, in accordance with this invention a linker can comprise an indirect means for associating the ligand with
25 the immunogen/cytotoxin/compound capable of altering macrophage function, such as by connection through spacer arms or bridging molecules. The chemistry of the linker can be designed to resist lysis or become susceptible to lysis following uptake by the targeted macrophage. Both direct and indirect means for association should not prevent the binding of the ligand to the receptor on the activated macrophages for
30 operation of the method of the present invention.

In the embodiment where the ligand is folic acid, an analog of folic acid, or any other folate receptor-binding molecule, the folate ligand can be conjugated to the immunogen, cytotoxin, or other compound capable of altering

macrophage function by an art-recognized procedure that utilizes trifluoroacetic anhydride to prepare γ -esters of folic acid via a pteroyl azide intermediate. This procedure results in the synthesis of a folate ligand, conjugated to the immunogen/cytotoxin/other compound capable of altering macrophage function only through the γ -carboxy group of the glutamic acid groups of folate. Alternatively, folic acid analogs can be coupled by art-recognized procedures through the α -carboxy moiety of the glutamic acid group or both the α and γ carboxylic acid entities.

Alternatively, the ligand conjugate can be one comprising a liposome wherein the targeted entity, such as the cytotoxin or other compound capable of altering macrophage function, is contained within a liposome which is itself covalently linked to the activated macrophage-binding ligand.

In accordance with one embodiment there is provided a method of treating lupus erythematosus by administering to a patient suffering from such disease state an effective amount of a composition comprising a conjugate of the formula L-X wherein L is as defined above and the group X comprises a cytotoxin, an immunogen, or another compound capable of altering macrophage function. Exemplary of cytotoxic moieties useful for forming conjugates for use in accordance with the present method include clodronate, anthrax, *Pseudomonas* exotoxin, typically modified so that these cytotoxic moieties do not bind to normal cells, and other toxins or cytotoxic agents including art-recognized chemotherapeutic agents such as p38 MAP kinase inhibitors, adrenocorticoids, alkylating agents, antiandrogens, antiestrogens, androgens, estrogens, antimetabolites such as cytosine arabinoside, purine analogs, pyrimidine analogs, and methotrexate, busulfan, carboplatin, chlorambucil, cisplatin and other platinum compounds, tamoxiphen, taxol, cyclophosphamide, plant alkaloids, prednisone, hydroxyurea, teniposide, and bleomycin, nitrogen mustards, nitrosureas, vincristine, vinblastine, inflammatory and proinflammatory agents, and the like. Such toxins or cytotoxic components can be directly conjugated to the activated macrophage binding moiety, for example, folate or other folate receptor binding ligands, or they can be formulated in liposomes which themselves are targeted as conjugates of macrophage binding ligands typically by covalent linkages to component phospholipids.

Similarly, when the group X comprises a compound capable of altering macrophage function, for example, a cytokine such as IL-10 or IL-11, the cytokine

can be covalently linked to the targeting moiety L, for example, a folate receptor-binding ligand or an antibody or antibody fragment, or the macrophage function altering cytokine can be encapsulated in a liposome which is targeted to activated macrophages by pendent macrophage targeting entities L covalently linked to one or
5 more phospholipid liposome components.

The method of the present invention can result in an elimination or reduction of activated macrophages or other stimulated immune cells (e.g., stimulated dendritic cells or stimulated macrophages distinct from normal resting tissue resident macrophages) or an inhibition of the activity of the activated/stimulated cells.

10 Treatment of symptomatic lupus erythematosus is contemplated in accordance with the invention. Also, prophylactic treatment to prevent return of the symptoms of lupus erythematosus is contemplated in accordance with this invention. The prophylactic treatment can comprise an initial treatment with the ligand conjugate, such as treatment in a multiple dose daily regimen, and one or more additional
15 treatments or series of treatments after an interval of days or months following the initial treatments(s).

In one embodiment, the group X in the activated macrophage targeted conjugate L-X comprises an immunogen, the ligand conjugates being effective to
20 "label" a population of activated macrophages in the patient suffering from lupus erythematosus for specific elimination by an endogenous immune response or by co-administered antibodies. The use of ligand conjugates wherein X is an immunogen in accordance with this embodiment results in an immune response-mediated elimination of an activated macrophage population. Such can be effected through an endogenous immune response or by a passive immune response effected by co-
25 administered antibodies. The endogenous immune response may include a humoral response, a cell-mediated immune response, and any other immune response endogenous to the patient, including complement-mediated cell lysis, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody opsonization leading to phagocytosis, clustering of receptors upon antibody binding resulting in signaling of
30 apoptosis, antiproliferation, or differentiation, and direct immune cell recognition of the delivered immunogen. It is also contemplated that the endogenous immune response will employ the secretion of cytokines that regulate such processes as the multiplication and migration of immune cells. The endogenous immune response

may include the participation of such immune cell types as B cells, T cells, including helper and cytotoxic T cells, macrophages, natural killer cells, neutrophils, LAK cells, and the like.

In another embodiment, the ligand conjugates, wherein X is an immunogen, can be internalized and the immunogen can be degraded and presented on the macrophage cell surface for recognition by immune cells to elicit an immune response directed against macrophages presenting the degraded immunogen.

The humoral response can be a response induced by such processes as normally scheduled vaccination, or active immunization with a natural antigen or an unnatural antigen or hapten, e.g., fluorescein isothiocyanate (FITC) or dinitrophenyl (DNP), with the unnatural antigen inducing a novel immunity. Active immunization can involve multiple injections of the natural or unnatural antigen or hapten scheduled outside of a normal vaccination regimen to induce the immunity. The humoral response can also result from an innate immunity where the patient has a natural preexisting immunity, such as an immunity to α -galactosyl or foreign blood groups.

Alternatively, a passive immunity can be established by administering antibodies to the patient such as natural antibodies collected from serum or monoclonal antibodies that may or may not be genetically engineered antibodies, including humanized antibodies. The utilization of a particular amount of an antibody reagent to develop a passive immunity, and the use of a ligand conjugate, wherein X is an immunogen and wherein the passively administered antibodies are directed to the immunogen, would provide the advantage of a standard set of reagents to be used in cases where a patient's preexisting antibody titer is not therapeutically useful. The passively administered antibodies can be "co-administered" with the ligand conjugate, and co-administration is defined as administration of antibodies at a time prior to, at the same time as, or at a time following administration of the ligand conjugate.

In the above-described embodiments, wherein X in the ligand conjugate of the formula L-X is an immunogen, it is contemplated that the preexisting antibodies, induced antibodies, or passively administered antibodies can be redirected to activated macrophages by preferential binding of the ligand conjugates to the activated macrophage cell populations, and such cells can be killed by complement-mediated lysis, ADCC, antibody-dependent phagocytosis, or antibody clustering of receptors. The cytotoxic process may also involve other types of immune responses,

such as cell-mediated immunity, as well as secondary responses that arise when the attracted antigen-presenting cells phagocytose the activated macrophages and present antigens of such cells to the immune system for elimination of other activated macrophages presenting such antigens.

5 Acceptable immunogens for use in preparing the conjugates used in the method of the present invention are immunogens that are capable of eliciting antibody production in the patient or that have previously elicited antibody production in the patient, resulting in a preexisting immunity, or that constitute part of the innate immune system. Alternatively, antibodies directed against the immunogen may be
10 administered to the host animal to establish a passive immunity. Suitable immunogens for use in the invention include antigens or antigenic peptides against which a preexisting immunity has developed via normally scheduled vaccinations or prior natural exposure to such agents such as polio virus, tetanus, typhus, rubella, measles, mumps, pertussis, tuberculosis and influenza antigens, foreign blood group
15 determinants, and α -galactosyl groups. In such cases, the ligand conjugates can be used to redirect a previously acquired humoral or cellular immunity to a population of activated macrophages in the patient for elimination of such cells.

Other suitable immunogens include antigens or antigenic peptides to which the patient has developed an immunity through immunization against a natural
20 or an unnatural antigen or hapten, for example, fluorescein isothiocyanate (FITC) or dinitrophenyl (DNP) and antigens against which an innate immunity exists, for example, superantigens and muramyl dipeptide. It is also contemplated that MHC I restricted peptides could be linked to the ligand for use in redirecting cellular immunity to macrophages and eliciting T cell killing of macrophages.

25 At least one additional therapeutic factor can be administered to the patient in combination with the above-detailed methodology to enhance the elimination of activated macrophages, or more than one additional therapeutic factor can be administered. The therapeutic factor can be selected from a compound capable of stimulating an endogenous immune response, a cytotoxin, or another therapeutic
30 factor capable of complementing the efficacy of the administered ligand conjugates. The method of the invention can be performed by administering to the patient, in addition to the above-described ligand conjugates, compounds or compositions capable of stimulating an endogenous immune response including, but not limited to,

cytokines or immune cell growth factors such as interleukins 1-18, stem cell factor, basic FGF, EGF, G-CSF, GM-CSF, FLK-2 ligand, HILDA, MIP-1 α , TGF- α , TGF- β , M-CSF, IFN- α , IFN- β , IFN- γ , soluble CD23, LIF, and combinations thereof.

Chemotherapeutic agents, which are cytotoxic themselves and can kill
5 or inhibit the activity of activated macrophages, suitable for use in the method of the invention in combination with the ligand conjugates include p38 MAP kinase inhibitors, adrenocorticoids, alkylating agents, antiandrogens, antiestrogens, androgens, estrogens, antimetabolites such as cytosine arabinoside, purine analogs, pyrimidine analogs, and methotrexate, busulfan, carboplatin, chlorambucil, cisplatin
10 and other platinum compounds, tamoxiphen, taxol, cyclophosphamide, plant alkaloids, prednisone, hydroxyurea, teniposide, antibiotics such as mitomycin C and bleomycin, nitrogen mustards, nitrosureas, vincristine, vinblastine, and any other art-recognized cytotoxic or chemotherapeutic agent. Alternatively, the ligand conjugates can be administered in combination with any other compound known to be useful for
15 treatment of lupus erythematosus such as chloroquine.

The additional therapeutic factor can be administered to the patient prior to, after, or at the same time as the ligand conjugates and the therapeutic factor can be administered as part of the same composition containing the ligand conjugates or as part of a different composition than the ligand conjugates. Any such therapeutic
20 composition containing an additional therapeutic factor at a therapeutically effective dose can be used in the method of the present invention. The therapeutic factor can be administered at a suboptimal dose along with the ligand conjugates in a combination therapy to avoid development of resistance to the therapeutic factor by the patient.

25 The amount of the ligand conjugate effective for use in accordance with the invention depends on many parameters, including the nature of the disease being treated, the molecular weight of the conjugate, its route of administration and its tissue distribution, and the possibility of co-usage of other therapeutic agents. The effective amount to be administered to a patient is typically based on body surface
30 area, patient weight and physician assessment of patient condition. In accordance with the invention an "effective amount" of the ligand conjugate is an amount sufficient to bind to activated macrophages or other stimulated immune cells and to be useful in the treatment of lupus erythematosus. The effective amount of the ligand

conjugate to be administered to a patient being treated for lupus erythematosus can range from, for example, about 1 ng/kg to about 10 mg/kg, or from about 10 μ g/kg to about 1 mg/kg, or from about 100 μ g/kg to about 500 μ g/kg.

Any effective regimen for administering the ligand conjugates can be used. For example, the ligand conjugates can be administered as single doses, or they can be divided and administered as a multiple-dose daily regimen. Further, a staggered regimen, for example, one to three days per week can be used as an alternative to daily treatment, and for the purpose of defining this invention such an intermittent or staggered daily regimen is considered to be equivalent to every day treatment and within the scope of this invention. In one embodiment, the patient is treated with multiple injections of the ligand conjugate to eliminate the population of activated macrophages. In one embodiment, the patient is treated, for example, injected multiple times with the ligand conjugate at, for example, at 12-72 hour intervals or at 48-72 hour intervals. Additional injections of the ligand conjugate can be administered to the patient at intervals of days or months after the initial injections, and the additional injections prevent recurrence of disease. Alternatively, the ligand conjugates can be administered prophylactically to prevent the occurrence of disease in patients known to be disposed to development of lupus erythematosus.

In one embodiment, more than one type of ligand conjugate can be used, for example, the patient can be pre-immunized with fluorescein isothiocyanate and dinitrophenyl and subsequently treated with fluorescein isothiocyanate and dinitrophenyl linked to the same or different targeting ligands in a co-dosing protocol. Alternatively, more than one type of ligand can be linked to one or more targeted entities (e.g., an immunogen) and conjugates with different ligands can be used in a co-dosing protocol.

The ligand conjugates administered in accordance with the method of this invention are preferably administered parenterally to the patient being treated for lupus erythematosus, for example, intravenously, intradermally, subcutaneously, intramuscularly, or intraperitoneally, in combination with a pharmaceutically acceptable carrier. Alternatively, the conjugates can be administered to the patient being treated for lupus erythematosus by other medically useful procedures such as in an orally available formulation. In accordance with the invention, a "patient being treated for lupus erythematosus" means any patient suspected of having lupus

erythematosus who would be predicted to benefit from the method of the present invention.

The conjugates used in accordance with this invention of the formula L-X are used in one aspect of this invention to formulate diagnostic compositions comprising effective amounts of the conjugate and an acceptable carrier therefor. Examples of parenteral dosage forms include aqueous solutions of the conjugate, for example, a solution in isotonic saline, 5% glucose or other well-known pharmaceutically acceptable liquid carriers such as alcohols, glycols, esters and amides. The parenteral compositions for use in accordance with this invention can be in the form of a reconstitutable lyophilizate comprising the one or more doses of the ligand conjugate. Any orally available dosage forms known in the art can also be used.

The ligand conjugates can also be delivered to a patient using an osmotic pump. In another aspect of the invention, the ligand conjugates can be formulated as one of any of a number of prolonged release dosage forms known in the art such as, for example, the biodegradable carbohydrate matrices described in U.S. Patents Nos. 4,713,249; 5,266,333; and 5,417,982, the disclosures of which are incorporated herein by reference.

20

EXAMPLE 1

Analysis of EC20 Uptake in Organs of Lupus Prone MRL/MpJ-*Tnfrsf6^{lpr}* Mice

Lupus prone MRL/MpJ-*Tnfrsf6^{lpr}* mice were used to determine whether binding of a folate-targeted ^{99m}Tc chelating chemical moiety (EC20; see International Publication No. WO 03/092742, incorporated herein by reference) could be detected in the organs of mice prone to lupus erythematosus. Lupus prone MRL/MpJ-*Tnfrsf6^{lpr}* mice have a mutation within the gene encoding the Tnfrsf protein (a member of the tumor necrosis factor family), and at 3 months of age exhibit greatly increased levels of circulating immune complexes and severe glomerulonephritis. Female MRL/MpJ-*Tnfrsf6^{lpr}* mice have a 17-week life span. A control group of mice, denominated MRL/MpJ mice, are control mice that have only mild glomerular lesions at 3 months of age, and females have a 73-week life span.

30

Female lupus prone MRL/MpJ-*Tnfrsf6^{lpr}* mice (n = 3/group) and female MRL/MpJ mice (n = 3/group) were purchased from Jackson Laboratories (Bar

Harbor, ME). The mice were placed on a folate deficient diet at the age of 12 weeks. Three weeks later the biodistribution of EC20 was evaluated in lupus prone and control mice. For the EC20 biodistribution studies, each mouse was administered with 1.8 mCi of ^{99m}Tc and 6.25×10^{-9} moles of EC20. The total injection volume for each mouse was 400 μl and the injections were i.p. The mice were sacrificed at 4 hours after injection, and organs were extracted for EC20 biodistribution analysis (i.e., the radioactivity per gram of tissue was measured (percentage injected dose of wet weight tissue (% ID/g)); see Fig. 1).

The results presented in Fig. 1 show that binding of ^{99m}Tc -EC20 was greater in all examined organs of lupus prone MRL/MpJ-*Tnfrsf6^{lpr}* mice compared to the organs of MRL/MpJ control mice. In particular, binding of ^{99m}Tc -EC20 was greater in the liver, spleen, duodenum, skin, and muscle of lupus prone MRL/MpJ-*Tnfrsf6^{lpr}* mice compared to control mice.

EXAMPLE 2

Effect of Folate-FITC Conjugates on Survival of Lupus Prone MRL/MpJ-*Tnfrsf6^{lpr}* Mice

Female lupus prone MRL/MpJ-*Tnfrsf6^{lpr}* mice were purchased from Jackson Laboratories (Bar Harbor, ME). Five-week old mice ($n = 9/\text{group}$) were immunized subcutaneously at multiple sites (2 sites in a 50 μl volume each) on day 0 with TiterMax Gold® adjuvant (in a 1:1 volume/volume ratio with folate-FITC conjugates) in combination with fluorescein isothiocyanate (FITC)-KLH conjugates (a total of 50 $\mu\text{g}/\text{mouse}$). Any effective adjuvant known in the art can be used. On day 20, the mice were placed on a folate deficient diet. On day 28 (at 9 weeks old) the mice were immunized a second time with TiterMax Gold® and fluorescein isothiocyanate (FITC)-KLH conjugates as described above.

After assuring that anti-FITC antibody titers were high in all mice (as evidenced by the results of ELISA assays of serum samples of the mice conducted on day 33), each animal was injected intraperitoneally at a single site with either phosphate buffered saline (PBS) or with 600 nmoles/kg of folate-FITC starting on day 35. For the next 25 weeks, the mice were injected daily with PBS or folate-FITC as described above.

The efficacy of this immunotherapy was then evaluated by monitoring survival as a function of time of folate-FITC treated mice compared to control mice (i.e., injected with PBS). As shown in Fig. 3, control mice began dying at week 17 and all control mice were dead by week 25. In contrast, all mice treated with folate-FITC were alive at week 25 after initiation of treatment with folate-FITC conjugates indicating the method of the present invention is a promising treatment for lupus erythematosus. Fig. 2 shows a photomicrograph of lupus prone MRL/MpJ-*Tnfrsf6*^{lpr} mice from this study that were either injected with folate-FITC (mouse on the left) or were injected with PBS (control; mouse on the right). This photomicrograph shows the striking difference in the health of lupus prone mice that have been treated with folate-FITC conjugates versus lupus prone control mice.

CLAIMS:

1. Use of a conjugate of the general formula
L-X
5 where the group L comprises a ligand capable of binding to activated macrophages and the group X comprises an immunogen, a cytotoxin, or another compound capable of altering macrophage function, in the manufacture of a medicament for treating lupus erythematosus.
2. The use of claim 1 wherein the group L comprises a vitamin, or
10 a vitamin-receptor binding analog or derivative thereof.
3. The use of claim 1 or 2 wherein L comprises a folate receptor binding ligand.
4. The use of claim 1 wherein L comprises an antibody or an activated macrophage binding antibody fragment.
- 15 5. The use of claim 1, 2, or 3 wherein the group X comprises an immunogen.
6. The use of claim 1, 2, or 3 wherein the group X comprises a cytotoxin.
7. The use of claim 6 wherein the group X further comprises a
20 liposome.
8. The use of claim 2 wherein the group X comprises a cytokine.

CONJUGATES AND USE THEREOF

ABSTRACT OF THE DISCLOSURE

5 The invention relates to a method of treating lupus erythematosus. In one embodiment, the method comprises the step of administering to a patient suffering from lupus erythematosus an effective amount of a composition comprising a conjugate or complex of the general formula



10 where the group L comprises a ligand capable of binding to activated macrophages or other stimulated immune cells, and the group X comprises an immunogen, a cytotoxin, or another compound capable of altering macrophage function. In another embodiment, the method comprises the step of administering to a patient suffering from lupus erythematosus an effective amount of a composition comprising a
15 conjugate of the general formula



where the group L comprises a vitamin, or a vitamin-receptor binding analog or derivative thereof, and the group X comprises an immunogen, a cytotoxin, or another compound capable of altering macrophage function.

20

1/3

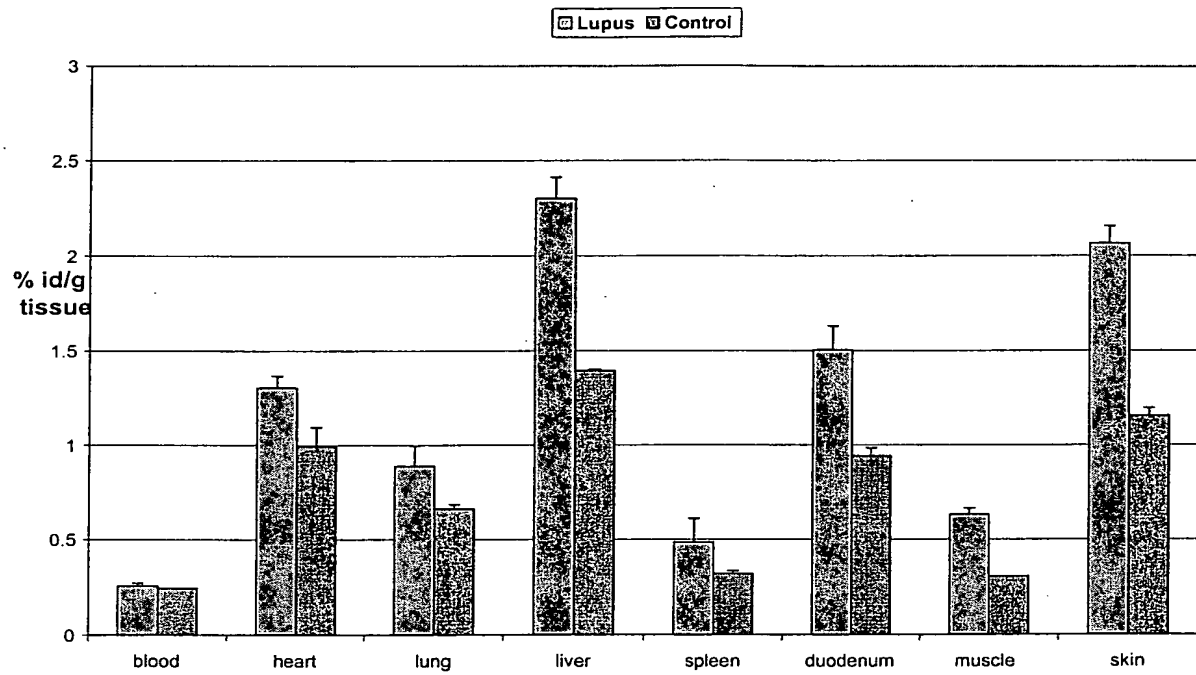


FIG. 1

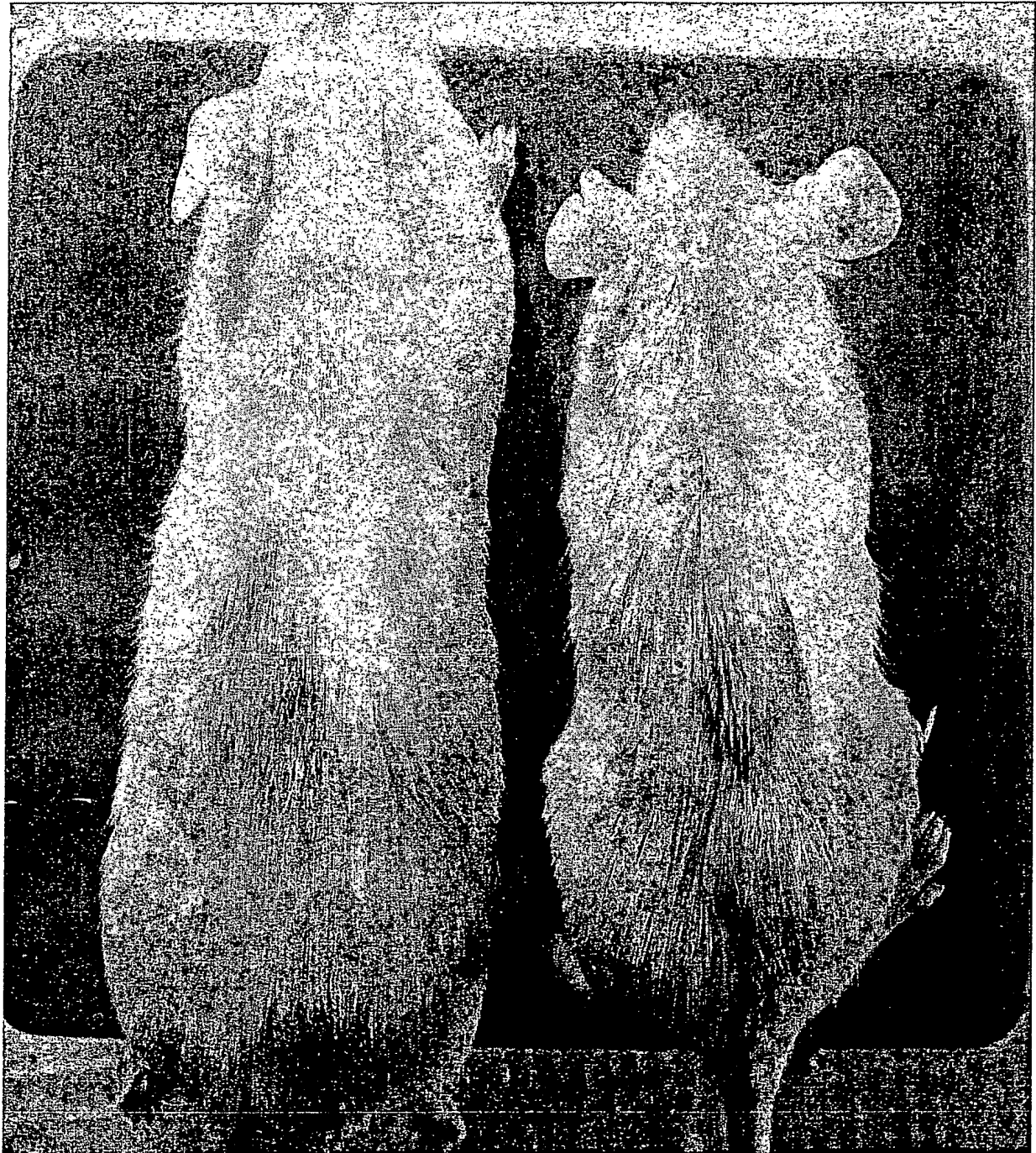


FIG. 2

3/3

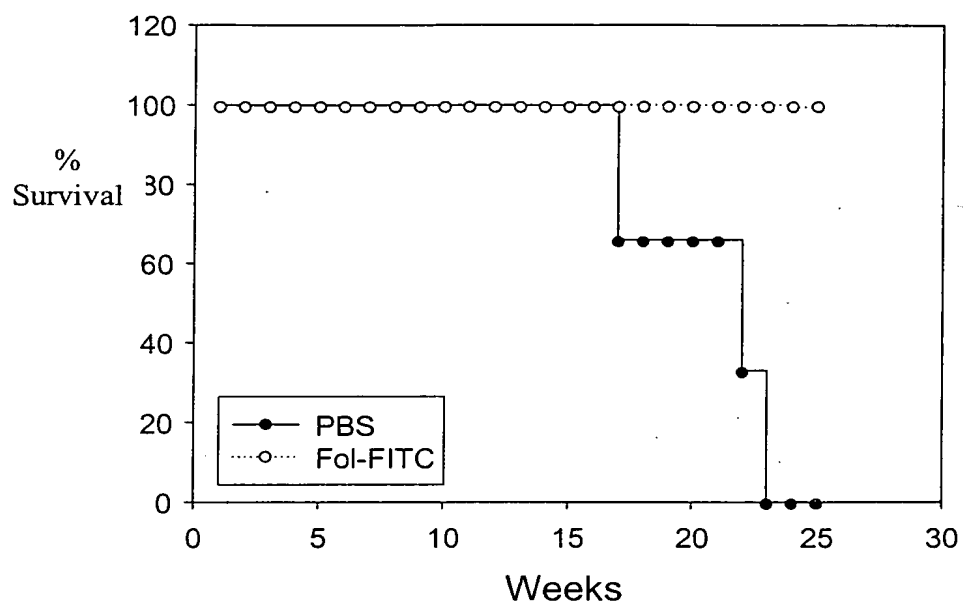


FIG. 3